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(54) Title: RECOMBINANT EQUINE HERPESVIRUS TYPE 1 (EHV-1) COMPRISING A DYSFUNCTIONAL GENE 71 REGION AND USE THEREOF AS A VACCINE

(57) Abstract

Vaccine formulation comprising EHV-1 gene 71 dysfunctional mutant and uses thereof.

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RECOMBINANT EQUINE HERPESVIRUS TYPE 1 (EHV-1) COMPRISING A DYSFUNCTIONAL GENE 71 REGION AND USE THEREOF AS A VACCINE

The present invention relates to a viral vaccine containing an attenuated EHV-1 virus comprising a gene deletion in the genome thereof, uses thereof and methods of treating EHV-1 related disease. In particular, the invention relates to a viral vaccine composition for use against Equine herpesvirus type 1 (EHV-1).

EHV-1 is a member of the subfamily alphaherpesvirinae and is a significant viral pathogen of horses. Clinical problems caused by EHV-1 include respiratory disease, abortion and neurological disorders (Bryans J.T., and Allen, G.P., Kluwer Academic Publishers, Norwell MA, 1989). As such, EHV-1 is responsible for significant economic losses within the equine industry.

The EHV-1 genome is a linear double-stranded DNA molecule of approximately 150 kbp in size which can be divided into two covalently linked components: the long and short regions. The long region consists of an unique sequence (U_L) flanked by a small inverted repeat (IR_L and TR_L). The short region comprises an unique sequence (U_S) flanked by a large inverted repeat (IR_S and TR_S).

EHV-1 occurs as pathogenic and non-pathogenic strains and recently, the complete DNA sequence of a pathogenic strain, Ab4, has been determined and the sequence has been deposited with the GenBank Library under Accession No. M 86664 (Telford, E.A.R. et al., Virology 189, pp. 304-316 (1990)).

The genome is 150,223 bp in size and contains 81 open reading frames predicted to encode polypeptides. The sizes of its components are U_L, 112,870 bp; TR_L/IR_L, 32 bp; U_S, 11,861 bp; and IR_S/TR_S, 12,714 bp. Interestingly, there are five genes, 1, 2, 67, 71, and 75, which have no homologues in any of the herpesviruses sequenced to date; i.e. they are unique to EHV-1.

Each of the genes 1, 2, 67, 71 and 75 is believed to encode a protein, however, the function of the individual proteins is unclear. Recently it has been demonstrated that the EHV-1 gene 71 product is involved in adsorption/penetration of virus and egress of virus from infected cell nuclei (Sun Y. et al., Journal of General Virology 77 pp. 493-500 (1996)).

The prior art does not teach or suggest the use of EHV-1 gene 71 deletion mutants comprising a dysfunctional gene 71 region in the manufacture and use of vaccines against EHV-1 related disease.

Control by vaccination of EHV-1 infection has been a long-sought goal. Current EHV-1 vaccines comprise chemically inactivated virus vaccines and modified live virus vaccines. Inactivated vaccines generally induce a low level of immunity and require additional immunisations and are expensive to produce. The use of such vaccines carries with it the risk that some infectious viral particles may survive the inactivation process and cause disease after administration to the animal.

In general, attenuated live virus vaccines are preferred because they evoke a longer-lasting immune response (often both humoral and cellular) and are easier to produce. Live attenuated EHV-1 vaccines are available which are based on live EHV-1 virus

attenuated by serial passage of virulent strains in tissue culture. However, serial passaging of virulent strains can give rise to uncontrolled mutations of the viral genome, resulting in a population of virus particles heterogeneous in their virulence and immunising properties. It is also known that such EHV-1 attenuated live virus vaccines can revert to virulence resulting in disease of the inoculated animals and the possible spread of pathogen to other animals.

The present inventors have now identified a suitable strain of live EHV-1 mutant virus comprising a dysfunctional region of the EHV-1 genome located within the short unique region thereof, which mutant may be used in a live EHV-1 vaccine formulation. Specifically, the inventors have found that EHV-1 mutants dysfunctional for production of a protein encoded by gene 71 can be used in a live EHV-1 vaccine formulation. Such mutants are shown to be substantially less virulent than wild type EHV-1 viruses. Furthermore, gene 71 has been found to be non-essential for EHV-1 growth in cell culture (Sun Y. and Brown S.M., Virology 199 pp. 448-452 (1994)). The inventors have also found that EHV-1 viruses comprising dysfunctional gene 71 regions of their genome are immunogenic. Such viruses are indicated for use as components in vaccine formulations or therapeutic compositions against EHV-1 infection. Accordingly, it is with EHV-1 viruses comprising a dysfunctional region located in the gene 71 protein coding region, and in particular between nucleotides 129,096 and 131,489 of the native genome which the present invention is concerned.

Statement of Invention

A first aspect of the present invention provides a vaccine formulation comprising a live recombinant EHV-1 virus modified so as to contain a dysfunctional gene 71 region located within the U_S region of the virus genome and a pharmaceutically acceptable carrier.

A "dysfunctional gene 71 region" is one which is substantially incapable of coding for the native polypeptide or a functional equivalent. Thus, a "dysfunctional gene 71 region" means that the gene 71 region has been modified by deletion, insertion or substitution (or other change in the DNA sequence such as by rearrangement) such that the gene 71 region does not express a native EHV-1 gene 71 polypeptide or a functionally equivalent product thereof. It is known that EHV-1 gene 71 encodes a 797 amino acid polypeptide and that the peptide is an O-linked 192 kDa glycoprotein (Sun, Y. et al., Journal of General Virology 75, pp. 3117-3126 (1994)). Thus, vaccine formulations comprising modified EHV-1 viruses of the invention may include viruses modified in one or more ways via recombinant DNA technology. Examples of the types of modifications which may be made include:

(i) A deletion of the entire gene 71 from the genome of an EHV-1 wild type virus. For example, a deletion of the nucleotide sequence from the wild type EHV-1 genome between about nucleotide 129,096 to about nucleotide 131,489.

(ii) A deletion of a portion of gene 71 from the genome of an EHV-1 wild type virus. A "portion of the gene 71" means a deletion which is sufficient to render any polypeptide encoded

by the gene 71 deletion mutant and expressed thereby substantially incapable of a physiological activity similar to that of the native polypeptide produced by wild type EHV-1. The deletion may be between 50% and 100% of the nucleotide sequence located between about nucleotides 129,096 and 131,489 of the wild type EHV-1 genome. The deletion may be from 70% to 100% of the gene 71 nucleotide sequence, or the deletion may be from about 70% to 90% of the gene 71 nucleotide sequence, for example, about 80% of the gene 71 nucleotide sequence.

(iii) The deletion of the or a portion of gene 71 as described in (i) and (ii) above will leave a "gap" in the EHV-1 genome corresponding to the gene 71 open reading frame (ORF) or a portion thereof. A suitable gene or genes may be inserted into the "gap" such as a marker gene. Suitable marker genes include but are not restricted to enzyme marker genes, for example the lac-Z gene from E.coli, antibiotic marker genes such as hygromycin, neomycin and the like. Such marker genes are commonly employed in the art. Generally, marker genes, if any, which may be employed in a gene 71 deletion mutant of the invention should be such so as to not cause substantial deleterious or long lasting side-effects to a recipient animal.

In a preferment, the "gap" made by the deletion of the or a portion of the gene 71 from a wild type EHV-1 virus is not filled with a gene insert, the cut ends of the two pieces of the genome being ligated together using conventional recombinant DNA technology. The skilled addressee will appreciate that the term "deletion mutant" encompasses those situations wherein the "gap" left by the partial or total deletion of gene 71 may be filled

with a gene insert, for example a marker gene or nonsense nucleotide sequence (i.e. a sequence incapable of giving rise to a protein or polypeptide product) or those situations wherein the gap is not filled by a heterologous or other nucleotide sequence. In such a case, the appropriate free ends of the two pieces of the genome are ligated together.

(iv) The deletion within the gene 71 region may comprise a deletion of a small number of nucleotides, for example 1, 2 or more nucleotides. Such deletions can be achieved using recombinant DNA technology. Thus, the translational ORF can be altered resulting in the production of a protein which lacks the physiological function of the gene 71 native polypeptide. The skilled addressee will also appreciate that such deletions in the translational ORF of gene 71 may also give rise to a dysfunctional gene 71 which is incapable of coding for a whole polypeptide, truncated peptide or even any peptide. Such proteins, if produced, generally lack the physiological functionality of the protein product of a normal gene 71 ORF.

(v) Nucleotide insertions can also be made in the EHV-1 gene 71 region using recombinant DNA technology which gives rise to dysfunctional gene 71 polypeptides substantially incapable of functional activity. For example, stop codons may be inserted into the gene 71 region, resulting in the production of non-functional fragments of the polypeptide encoded by native gene 71.

The skilled addressee will appreciate that such nucleotide insertions can be of any length from 1 or more nucleotides to a number of nucleotides making up, for example, nonsense nucleotide

sequences which can have the effect of altering the translational ORF resulting in the non-production of a polypeptide or indeed, the production of a protein lacking the physiological function of the gene 71 native polypeptide. The skilled addressee will also appreciate that such insertions in the translational ORF of gene 71 may also give rise to a dysfunctional gene 71 which is incapable of coding for a whole polypeptide, truncated peptide or even any peptide. Such proteins, if produced, generally lack physiological functionality.

Naturally, the skilled addressee will appreciate that gene 71 deletions and insertions from non-wild type EHV-1 viruses as outlined above are encompassed by the present invention.

In a preferment there is provided a vaccine formulation comprising a live recombinant attenuated immunogenic EHV-1 gene 71 deletion mutant virus and a pharmaceutically acceptable carrier.

In a second aspect of the invention there is provided a live, recombinant EHV-1 comprising a dysfunctional gene 71 region for use as a vaccinating agent or in a vaccine formulation. Preferably, there is provided a live, recombinant, attenuated immunogenic EHV-1 gene 71 deletion mutant virus for use as a vaccinating agent or in a vaccine formulation.

The live, recombinant EHV-1 may optionally include an inserted gene positioned at the gene 71 locus in lieu of a substantial portion of gene 71 or the whole of gene 71.

Generally, the vaccine or vaccine formulation is not used on non-pregnant animals because it can give rise to abortigenesis.

In a third aspect of the invention there is provided the use of a live, recombinant EHV-1 virus for producing antibodies or cell mediated immunity to EHV-1 which comprises a dysfunctional gene 71 region located within the U_S region of the virus genome for the manufacture of an EHV-1 vaccine for the prophylaxis and/or treatment of EHV-1 infection. Preferably, there is provided use of a live, recombinant, attenuated immunogenic EHV-1 gene 71 deletion mutant virus for the manufacture of an EHV-1 vaccine for the prophylaxis and/or treatment of EHV-1 infection. Most preferably, the use is in horses.

In a fourth aspect of the invention there is provided a method of treating animals which comprises administering thereto a vaccine composition comprising a live, recombinant EHV-1 virus modified so as to contain a dysfunctional gene 71 region located within the U_S region of the virus genome to animals in need thereof. Preferably, the animals are horses. Preferably still, the method of treating animals comprises administering a vaccine composition comprising a recombinant, live, attenuated, immunogenic EHV-1 gene 71 deletion mutant virus to animals in need thereof. Naturally, the vaccine formulation may be formulated for administration by oral dosage (e.g. as an enteric coated tablet), by parenteral injection or otherwise.

The invention also provides a process for preparing a live modified EHV-1 virus vaccine, which process comprises admixing a virus according to the invention with a suitable carrier or adjuvant.

For the preparation of a live attenuated vaccine, standard methodology may be used.

The mode of administration of the vaccine of the invention may be by any suitable route which delivers an immunoprotective amount of the virus of the invention to the subject. However, the vaccine is preferably administered parenterally via the intramuscular or deep subcutaneous routes. Other modes of administration may also be employed, where desired, such as oral administration or via other parenteral routes, i.e., intradermally, intranasally, or intravenously.

Generally, the vaccine will usually be presented as a pharmaceutical formulation including a carrier or excipient, for example an injectable carrier such as saline or apyrogenic water. The formulation may be prepared by conventional means.

The appropriate immunoprotective and non-toxic dose of such a vaccine can be determined readily by those skilled in the art, i.e., the appropriate immunoprotective and non-toxic amount of the virus contained in the vaccine of this invention may be in the range of the effective amounts of antigen in conventional whole virus vaccines. It will be understood, however, that the specific dose level for any particular recipient animal will depend upon a variety of factors including age, general health, and sex; the time of administration; the route of administration; synergistic effects with any other drugs being administered; and the degree of protection being sought. Of course, the administration can be repeated at suitable intervals if necessary.

Embodiments of the invention will now be illustrated by way of the following Figures and Examples.

Claims

CLAIMS 1. Vaccine formulation comprising a live, recombinant EHV-1 virus modified so as to contain a dysfunctional gene 71 region located within the Us region of the virus genome and a pharmaceutically acceptable carrier.

2. A vaccine formulation according to claim 1 comprising a live, recombinant, attenuated immunogenic EHV-1 gene 71 deletion mutant virus and a pharmaceutically acceptable carrier.

3. A vaccine formulation according to claim 1 or claim 2 wherein the dysfunctional gene 71 region of the recombinant EHV-1 virus comprises a deletion of at least one nucleotide between nucleotide 129,096 and nucleotide 131,489 of a wild type EHV-1 genome.

4. A vaccine formulation according to any one of claims 1 to 3 wherein the recombinant EHV-1 comprises a marker gene.

5. A live, recombinant EHV-1 comprising a dysfunctional gene 71 region for use as a vaccinating agent.

6. A live, recombinant, attenuated immunogenic EHV-1 gene 71 deletion mutant virus for use as a vaccinating agent.

7. Use of a live, recombinant, EHV-1 gene 71 deletion mutant virus in the manufacture of an EHV-1 vaccine for the prophylaxis and/or therapy of EHV-1 infection.

8. A method of treating an animal which comprises administering to an animal a vaccine composition comprising a live, recombinant EHV-1 virus modified so as to contain a dysfunctional gene 71 region located within the Us region of the virus genome.

9. A method according to claim 8 wherein the animal is a horse.

10. A method according to claim 8 or claim 9 wherein the vaccine composition comprises a recombinant, live, attenuated, immunogenic EHV-1 gene 71 deletion mutant virus.